Case Report

Esophageal Squamous Cell Carcinoma with Lymphoid Stroma: Histopathological and Immunohistochemical Analysis

Osamu Chino1), Hiroyasu Makuuchi1), Soji Ozawa1), Hideo Shimada1), Takayuki Nishi1), Yoshifumi Kise1), Tomoko Hanashi1), Soichiro Yamamoto1), Tadashi Hara1), Akihito Kazuno1), Hiroshi Kajiwara2), Kyoji Ogoshi1), Seiei Yasuda1)

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Abstract

Prominent lymphocytic infiltration and lymphoid follicles surrounding tumor cells are extremely rare findings in esophageal carcinoma. We report on the endoscopic, histological, and immunohistochemical features of a rare case of squamous cell carcinoma of the esophagus with lymphoid stroma. A 59-year-old woman was diagnosed with type 0-Is superficial esophageal carcinoma and underwent radical esophagectomy with lymph node dissection. Macroscopically, the tumor was protruding, and was covered with normal epithelium resembling a submucosal tumor. Histopathological examination demonstrated that the esophageal lesion was classified as a poorly differentiated squamous cell carcinoma with lymphoid stroma, extending to the deep submucosa (SM3) with lymph node metastasis (T1b, N2, M0, stage II). Epstein-Barr virus infection was ruled out by immunohistochemical and in situ hybridization analyses. Infiltrating B-lymphocytes were observed forming lymphoid follicles adjacent to carcinoma cell nests, and numerous T lymphocytes were widely spread throughout the specimen, as well as distributed in the marginal zone of the lymphoid follicles. Prominent human leukocyte antigen DR region (HLA-DR) immunoreactivity was noted in most carcinoma cells and focally infiltrating B cells in the lymphoid follicles, and these observations were thought to be due to activation of immunological interactions between carcinoma cells positive for HLA-DR and host lymphocytes.

Key Words: esophageal cancer, squamous cell carcinoma, lymphoid stroma, immunohistochemistry, HLA-DR.

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Introduction

Squamous cell carcinoma is the most common malignancy of the esophagus1), and although lymphoid stroma of neoplastic lesions has been reported in different organs such as the breast, nasopharynx, uterine cervix and stomach, prominent lymphocytic infiltration around tumor cells is extremely rare in esophageal carcinoma2-8). Lymphocytic infiltration is thought to represent a host immune reaction against tumor cells, and its prognostic significance has been well documented in human cancers9-13). Herein, we present a rare case of esophageal squamous cell carcinoma with lymphoid stroma, and describe its endoscopic, histological, and immunohistochemical characteristics.

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Case Report

During a medical check-up, a 59-year-old woman underwent endoscopic examination, which revealed a protruding lesion measuring 1.5 cm on the right-posterior wall of the middle thoracic esophagus. The patient was referred to Tokai University Hospital on October 28, 2005. She was asymptomatic, and physical examination at admission did not reveal any abnormalities. All laboratory data were within the normal ranges except for slight anemia, and the squamous cell carcinoma antigen and carcinoembryonic antigen levels, which were <0.5 ng/ml and 5.6 ng/ml, respectively.

Double-contrast esophagography demonstrated a protruding lesion with a slight barium fleck. The tumor (longitudinal size, 1.5 cm) was located on the posterior wall of the middle thoracic esophagus (Fig.1A, B). Endoscopic examination revealed a protruding tumor with a central depression, covered by normal-appearing esophageal epithelium stained with iodine but not with toluidine blue, resembling a submucosal tumor, 30 cm
Esophageal carcinoma with lymphoid stroma

from the incisor teeth (Fig. 2A, B). Endoscopic ultrasound demonstrated a hypoechoic lesion within the submucosal layer. Pathological examination of the preoperative biopsy material revealed uniform lymphocytic infiltration around a poorly differentiated squamous cell carcinoma, and the tumor was accordingly diagnosed as squamous cell carcinoma with lymphoid stroma.

The preoperative diagnosis was type 0-Ia\(^2\) superficial esophageal carcinoma invading the submucosa. Standard subtotal esophagectomy using a thoraco-laparotomy with three-field lymph node dissection was performed on December 6, 2005. The postoperative course was uneventful and the patient was discharged three weeks later. After the operation, the patient received two courses of adjuvant chemotherapy of cisplatin and 5-fluorouracil. At the lastest follow-up, (eight years and six months post-surgery) the patient was alive without tumor recurrence.

Fig. 1 Double contrast esophagography revealed the protruding lesion with a slight barium fleck. The size was 1.5 cm longitudinally, and the tumor was located on the right-posterior wall of the middle thoracic esophagus.
A: right anterior oblique view.
B: left anterior oblique view.

Fig. 2 A: Conventional endoscopic appearance of the tumor is protruding type with a slight central depression, covered by normal-appearing esophageal epithelium, mimicking a submucosal tumor, on the right-posterior wall of the middle thoracic esophagus.
B: Chromoendoscopic view showed the esophageal tumor stained with iodine.
Material and Methods

Esophageal tissue specimens
The resected materials were fixed by formalin. The whole tumor was cut and embedded in a paraffin block 3 mm in width, and subsequently, used for histopathological examination. Representative sections were also used for immunohistochemical analysis and in situ hybridization.

Immunohistochemical analyses
Epithelial markers analyzed immunohistochemically included keratin (Keratin-WSS) and epithelial membrane antigen (EMA). Leukocyte common antigen (LCA; CD45) was specific and reliable marker for lymphocytes. MT-1 (CD43), UCHL-1 (CD45RO), OPD4 (CD4) and CD8 were markers for T-lymphocyte expression respectively. MB-1 (CD45RA), L-26 (CD20) and CD79a are markers for B-lymphocyte expression respectively. Moreover, immunoreactivity against the DR region of human leukocyte antigen (HLA-DR), which is known to be closely associated with prominent lymphocytic infiltration in carcinoma cells 13 was assessed, and Epstein-Barr virus (EBV) infection in the tumor cells was evaluated by examination for EBV-encoded small RNA1 (EBER-1) by in situ hybridization, and examined by immunohistochemical technique using LMP-1.

Results
In the resected specimen, an elevated tumor measuring 1.5 × 1.3 cm, occupying approximately one-fourth of the circumference of the esophageal lumen was noted. The tumor showed expansive growth and a smooth surface, mimicking a submucosal tumor macroscopically.

Histopathological examination demonstrated that the esophageal lesion was classified as poorly differentiated squamous cell carcinoma extending into the deep submucosa (Fig. 3A). Metastatic carcinoma was found in the dissected lymph node along the gastric lesser curvature, and cancer cells were detected in one of 77 dissected lymph nodes from the abdomen. The infiltrating pattern was invasive and neither lymphatic nor blood vessel invasion was observed. According to the Japanese guidelines for clinical and pathologic studies on carcinoma of the esophagus, the tumor was classified as stage II (T1b, N2, M0) 13.

The lesion displayed marked lymphocytic infiltration and lymphoid follicle formation in the tumor stroma (Fig. 3B). The results of the immunohistochemical staining for the carcinoma cells and the infiltrating lymphocytes are summarized in Table 1. The carcinoma cells were uniformly positive for epithelial markers, such as Keratin-WSS and EMA, and HLA-DR immunoreactivity was noted in most of the carcinoma cells and focally infiltrating B-cells in the lymphoid follicles. The infiltrating lymphocytes were stained positively for LCA (CD45) consisting of numerous T-lymphocytes and small number of B-lymphocytes. The T-lymphocytes, which were positive for T-cell markers (MT-1 and UCHL-1), were found to be widely distributed in the specimens, as well as in the marginal zone of the lymphoid follicle. The majority of the T-cells displayed CD-8 immunoreactivity. Most of the B-lymphocytes forming lymphoid follicles adjacent to the carcinoma cell nests were positive for the B-cell markers (MB-1, CD20 and CD79a) (Fig. 4A-D).

Epstein-Barr virus (EBV) infection was ruled out by immunohistochemical and in situ hybridization analyses in the present case.

Discussion
Lymphoepithelioma-like carcinoma or medullary carcinoma has been reported in several organs, including the breast, nasopharynx, uterine cervix, and stomach. In Japan, squamous cell carcinoma is the most common malignancy of the esophagus. However, prominent lymphocytic infiltration is extremely rare in esophageal squamous cell carcinoma 15. This present case was unique in the prominent stromal lymphocytic infiltration around the carcinoma cells. To our knowledge, only 19 cases of the esophageal squamous cell carcinoma with lymphoid stroma have been reported, and interestingly, most of these cases occurred in Japanese patients. In these previous studies, the clinical features, such as age distribution, sex ratio and location of the lesion, showed no significant differences between esophageal carcinoma with lymphoid stroma and common esophageal cancers 15, 16. Thus, we speculate that squamous cell carcinoma with lymphoid stroma might be associated with etiological factors 16. However, these have been not clarified yet. Several investigators have reported an association between EBV infection and carcinoma with marked lymphocytic infiltration 15, 16. In this case and in the previous studies, however, EBV infection was ruled out by immunohistochemical and in situ hybridization analyses 13, 14.

Squamous cell carcinomas with lymphoid stroma commonly show protrusions and central depression covered by non-neoplastic epithelium, mimicking submucosal tumors both endoscopically and macroscopically. The present case was classified as T1b cancer limited to the submucosa, and showed preservation of the morphological characteristics of superficial carcinoma. The lymphoid stroma with lymphoid follicle formation was thought to arise in the submucosa during the early stage of cancer progression, and this tumor might have become a protruding-type tumor with predominantly subepithe-
Esophageal carcinoma with lymphoid stroma

**Table 1** Immunohistochemical staining of the esophageal carcinoma with lymphoid stroma

<table>
<thead>
<tr>
<th>Marker</th>
<th>Carcinoma cells</th>
<th>Lymphocytes</th>
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<tbody>
<tr>
<td></td>
<td>Keratin (Ker-WSS)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>EMA</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>LCA (CD45)</td>
<td>-</td>
</tr>
<tr>
<td>T cell</td>
<td>MT-1 (CD43)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>UCHL-1 (CD45RO)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>OPD4 (CD4)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CD8</td>
<td>-</td>
</tr>
<tr>
<td>B cell</td>
<td>MB-1 (CD45RA)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>L-26 (CD20)</td>
<td>-</td>
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<tr>
<td></td>
<td>CD79a</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HLA-DR</td>
<td>+</td>
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<tr>
<td></td>
<td>EBV</td>
<td></td>
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<tr>
<td></td>
<td>LMP-1</td>
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<td></td>
<td>EBER-1 (ISH)</td>
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EMA, epithelial membrane antigen. LCA, leukocyte common antigen. EBV, Epstein-Barr virus. ISH, in situ hybridization.

Fig. 3 Photomicrographs showing microscopic findings of esophageal squamous cell carcinoma with lymphoid stroma.
A: The main lesion showed poorly differentiated squamous cell carcinoma invading to the deep submucosal layer of the esophagus (original magnification ×20, H&E).
B: The tumor showed poorly differentiated squamous cell carcinoma with lymphoid stroma (original magnification ×100, H&E).

The favorable prognostic significance associated with this type of tumors, as previously describe for breast medullary carcinoma and gastric carcinoma with lymphoid stroma, appear to be an intrinsic feature, and accordingly our patient is surviving without postoperative recurrence.

Several investigators have summarized the features of infiltrating lymphocytes in human cancers. Previous studies have demonstrated that HLA-DR immunoreactivity in certain carcinomas is closely associated with prominent lymphocytic infiltration. In the present case, HLA-DR was highly expressed on most carcinoma cells, as well as on the focally infiltrating B-cells of lymphoid follicles.
We speculate that the esophageal carcinoma cells can activate T-cells mediated by cytokines. Accordingly, in the present case, T-lymphocytes were found around the lymphoid follicles, and most of these T-cells were positive for CD8, suggesting that they were natural killer cells. These findings suggest a host immunologic interaction against carcinoma cells positive for HLA-DR. HLA-DR-positive carcinoma cells appear to induce activation of T-cells. Moreover, we speculate that the infiltrating T-lymphocytes may have activated the B-lymphocytes, which subsequently formed the numerous secondary lymphoid follicles adjacent to the carcinoma cell nests.

In summary, we here reported a rare case of esophageal squamous cell carcinoma associated with prominent lymphocytic infiltration and lymphoid follicles, resembling medullary carcinoma. Endoscopically, the tumor was characterized by protruding-type appearance, and was covered with non-neoplastic epithelium. Prominent HLA-DR immunoreactivity was observed in the carcinoma cells. These observations may be due to activation of various immunological interactions between carcinoma cells positive for HLA-DR and the host lymphocytes, and further studies and reports of similar cases are required.

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References


